SID 16
(3 hits this paper)

RESULT 2
BTU51820
LOCUS BTU51820 376 bp DNA MAM 16-MAY-1996
DEFINITION Bos taurus Hereford 7 mitochondrial hypervariable region D-loop, partial sequence.
ACCESSION U51820
VERSION U51820.1 GI:1314616
KEYWORDS .
SOURCE cow strain=Hereford.
ORGANISM Mitochondrion Bos taurus
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REFERENCE 1 (bases 1 to 376)
AUTHORS Bradley,D.G., MacHugh,D.E., Cunningham,P. and Loftus,R.T.
TITLE Mitochondrial diversity and the origins of African and European cattle
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 93 (10), 5131-5135 (1996)
MEDLINE 96209869
REFERENCE 2 (bases 1 to 376)
AUTHORS Bradley,D.G., MacHugh,D.E., Cunningham,P. and Loftus,R.T.
TITLE Direct Submission
JOURNAL Submitted (19-MAR-1996) Daniel G. Bradley, Genetics, Trinity College Dublin, Dublin, 2, Ireland
FEATURES Location/Qualifiers
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ORIGIN

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Db    124 AGATCACGAGCTTAATTACCATGCCGCGTGAAA 156
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sequencer. Consensus sequences were constructed and analyzed with the help of the University of Wisconsin GCG software package. The BLAST algorithm from the National Center for Biotechnology Information (NCBI) was also employed for nucleotide and amino acid sequence homology search (Altschul, S. F. et al. (1990) *J. Mol. Biol.* 215:403–410).

Preparation of Recombinant Proteins:

The recombinant CR8 protein with histidine-tag was prepared using the XpressTM System (Invitrogen) according to the manufacturer's protocol. cDNA corresponding to the CR8 bHLH domain was obtained by PCR. The sequences of the primers, 5'-GGGGTCTACCAGGGATGTAC-3' (SEQ ID NO:15) for the 5' side, and 5'-GTAAACCACTCTGCAGGGCAATGA-3' (SEQ ID NO:16) for the 3' side, were slightly different from the final consensus sequence for CR8, but the difference did not affect the core bHLH motif. The PCR product was cloned into pT7Blue T-vector (Novagen) and subsequently into pRSET-A vector at BamHI and HindIII sites. Constructs were confirmed by DNA sequencing. The protein was overexpressed in JM109 at 37° C. in the presence of isopropylthio-b-D-galactoside (IPTG) by infecting the bacteria with M13 phages that contain the T7 RNA polymerase gene. The cells were lysed with 100 fg/ml lysozyme in native binding buffer (20 mM sodium phosphate, pH 7.8, 500 mM NaCl), the lysate was loaded on a ProBondTM Ni2+column, and the recombinant protein was eluted with native-imidazole elution buffer (20 mM sodium phosphate, pH 6.0, 500 mM NaCl, 500 mM imidazole). The protein was then dialyzed against lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 0.1% Triton X-100, 1 mM DTT) and concentrated on a Microcon 10 (Amicon). To confirm the purity and the quantity, the protein was fractionated on a 12% SDS-polyacrylamide gel with protein standards of known concentrations and visualized by silver staining. The recombinant protein corresponding to the bHLH domain of da was supplied by Dr. Michael Caudy (Cornell University Medical College).

Mobility Shift Assay:

The oligonucleotide probes used for the electrophoretic mobility shift assay (EMSA) are as follows: the Class A site used was the CACCTG hexamer (CAGGTG for the opposite strand) from the T5 promoter region of the Drosophila AS-C (Villares, R. et al. (1987) *Cell* 550:415–424) (5'-GATCGTAGTCACGCAGGTGGGATCCCTA-3' (SEQ ID NO:17) and 5'-GATCTAGGGATCCCACCTGCGTGACTAC-3' (SEQ ID NO:18) for the opposite strand), the Class B site was the CACGTG hexamer from the USF binding site in the adenovirus major late promoter (Gregor, P. D. et al. (1990) *Genes & Dev.* 4:1730–1740) (5'-GATCGGTGTAGGCCACGTGACCGGGTGT-3' (SEQ ID NO:19) and 5'-GATCACACCCGGTCACGTGGCCTACACC-3') (SEQ ID NO:20), the Class C site was the CACGCG hexamer (CGCGTG for the opposite strand) from the AS-C T5 promoter (5'-GATCGGCAGCCGGCACGCGACAGGGCC-3' (SEQ ID NO:21) and 5'-GATCGGCCCTGTCGCGTGCCGGCTGCC-3') (SEQ ID NO:22), and the N-box (CACNAG) was the double hexamer sequence from the Enhancer of split [E(spl)] m8 promoter (Klimbt, C. et al. (1989) *EMBO J.* 8:203–210) (5'-GATCACGCCACGAGCCACAAGGATTG-3' (SEQ ID NO:23) and 5'-GATCCAATCCTTGTGGCTCGTGGCGT-3' (SEQ ID NO:24). One strand of the oligonucleotide was labeled with [g-32P]ATP by T4 polynucleotide kinase, hybridized with three times excess of the opposite strand, and purified using MERmaid oligonucleotide purification kit (BIO 101). 150 ng of the protein was allowed to bind to 50,000 cpm (equivalent to 0.5 ng in a typical experiment) of the labeled probe for 15 min at room temperature in 20 mM Hepes, pH 7.6, 50 mM KCl, 10 mM DTT, 5% glycerol, 0.5 mM EDTA and 0.3 mg/ml BSA. Two microgram of poly (dI-dC) was added to each 20 fl reaction as on-specific DNA. Samples were analyzed on a 5% native polyacrylamide gel and visualized by autoradiography.

Regulation of CR8:

CR genes were originally defined in IL-2 stimulated normal human T cells. To examine CR8 expression in cytokine-dependent cell lines, the level of CR8 expression was measured by Northern hybridization in the IL-2-dependent human T cell line Kit 225, the IL-3-dependent mouse pro-B cell line Ba/F3, and the IL-2-dependent mouse T cell line CTLL2. The results of this experiment are illustrated in FIGS. **13A–13C**. In FIG. **13A**, RNA was isolated from quiescent normal human T cells (lanes 1 and 2), IL-2-dependent human T cell line Kit 225 (lanes 3 and 4), IL-3-dependent mouse pro-B cell line Ba/F3 (lanes 5 and 6) and IL-2-dependent mouse T cell line CTLL2 (lanes 7 and 8) left untreated (lanes 1, 3, 5, and 7) or stimulated with 500 pM recombinant human IL-2 (lanes 2, 4, and 8) or 10 U/ml recombinant mouse IL-3 (lane 6) for 2 hr at 37° C. The amount of the growth factor used in the experiment was sufficient to induce maximal [^{3}H]thymidine incorporation. Ten microgram of total RNA was analyzed on formaldehyde/agarose gel and hybridized with either human (lanes 1 to 4) or mouse (lane 5 to 8) CR8 cDNA.

As shown in FIG. **13A**, a single 3.2 kb species hybridized to the cDNA probe, and in all three cell lines tested, the level of CR8 was clearly augmented when the cells were stimulated with their respective growth factors. Correlation between the level of CR8 and that of DNA synthesis was in the presence of growth-inhibitory agents was also examined. In this regard, increases in cytoplasmic cAMP are known to inhibit the growth of many cell types, including lymphocytes (Johnson, K. W. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:6072–6076). IFNs also exert antiproliferative activity on many cell types (Pestka, S. et al. (1987) *Annu. Rev. Biochem.* 56:727–777). Therefore, Kit 225 was stimulated with IL-2, IFN β, or forskolin, which increases cytoplasmic cAMP by activating adenylate cyclase, either alone or in combination. IL-2-dependent [^{3}H]thymidine incorporation was inhibited by IFNb and forskolin in Kit 225 cells in a dose-dependent fashion (FIG. **13B**). FIG. **13B** demonstrates that IFNP and forskolin inhibit IL-2-dependent [^{3}H] thymidine incorporation by Kit 225 cells. Ten thousand quiescent Kit 225 cells were incubated with indicated reagents in 200 fl for 24 hr at 37° C. The culture was pulsed with [^{3}H]thymidine for the last 4 hr to monitor the DNA synthesis. (●), IL-2 only (500 pM); (◇), IL-2 500 pM+varying concentrations of IFNb (U/ml); (○), IL-2 500 pM+varying concentrations of forskolin (fM). While forskolin was capable of reducing the IL-2-dependent [^{3}H] thymidine incorporation almost to the basal level, IFNβ-mediated inhibition never exceeded 70% of the maximal incorporation in several independent experiments. The expression of CR8 was compared with that of c-myc, an IL-2-inducible immediate-early gene that encodes a bHLH protein and is implicated for cell proliferation (Marcu, K. B. et al. (1992) *Annu. Rev. Biochem.* 61:809–860).

FIG. **13C** shows the effect of antiproliferative agents on the expression of CR8 and c-myc transcripts in Kit 225.

5104

RESULT 7
BTU51824
LOCUS BTU51824 389 bp DNA MAM 16-MAY-1996
DEFINITION Bos taurus Jersey 5 mitochondrial hypervariable region D-loop, partial sequence.
ACCESSION U51824
VERSION U51824.1 GI:1314620
KEYWORDS .
SOURCE cow strain=Jersey.
ORGANISM Mitochondrion Bos taurus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Cetartiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE 1 (bases 1 to 389)
AUTHORS Bradley,D.G., MacHugh,D.E., Cunningham,P. and Loftus,R.T.
TITLE Mitochondrial diversity and the origins of African and European cattle
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MEDLINE 96209869
REFERENCE 2 (bases 1 to 389)
AUTHORS Bradley,D.G., MacHugh,D.E., Cunningham,P. and Loftus,R.T.
TITLE Direct Submission
JOURNAL Submitted (19-MAR-1996) Daniel G. Bradley, Genetics, Trinity College Dublin, Dublin, 2, Ireland
FEATURES Location/Qualifiers
source 1. .389
/organism="Bos taurus"
/organelle="mitochondrion"
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ORIGIN

Query Match 100.0%; Score 46; DB 3; Length 389;
Best Local Similarity 100.0%; Pred. No. 1.8e-08;
Matches 46; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 202 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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